

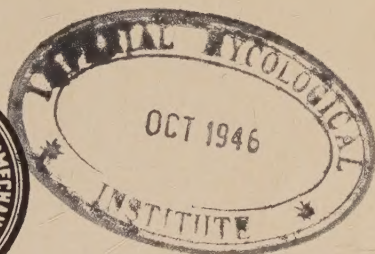
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Mycorrhizae and Phosphorus Nutrition of Pine Seedlings in a Prairie Soil Nursery

By A. L. McComb

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
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FORESTRY SECTION



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Mycorrhizae and Phosphorus Nutrition of Pine Seedlings in a Prairie Soil Nursery¹

By A. L. McComb²

The relation of the mycorrhizal soil fungi to the growth of conifers has long been a controversial subject.³ Recent experiments in nursery production, in afforestation and in extending the range of certain species of conifers (11, 14, 21, 32, 36, 37, 38) have reemphasized the importance of these fungi in promoting tree growth and aiding nutrition. Nonetheless, the manner in which these organisms work is still obscure, and growth stimulation of seedlings has been attributed both to effects resulting from the invasion of short roots by fungi and to the activity of the fungus in the soil adjacent to the roots.

The invasion of the short roots by the fungus commonly results in the formation of ectotrophic mycorrhizae which are characterized by an external fungal mantle and an internal Hartig net (16). When the fungus is more active, intracellular haustoria (48) are formed in addition to the intercellular net, and these types of mycorrhizae have been called ectendotrophic. The nature of the fungal infection is apparently dependent to a considerable degree upon the species of fungus and the relative vigor of both the host plant and the fungus. Endotrophic mycorrhizae, which are characterized by intracellular infection, but which lack the fungal mantle and Hartig net, are apparently not common among the pines.

When mycorrhizae are formed, the root system of the plant is often enlarged. According to Hatch (15) this enlargement results in better mineral nutrition. Better nutrition is derived directly from the greater number of absorbing short roots, the greater surface area of individual mycorrhizal roots, the delay in suberization of the cortex and endodermis, and the great extension of the fungal mycelium through the soil. Mycorrhizae are also claimed to enable trees to acquire an increased supply of any limiting

¹Part of a thesis entitled "Nutrition and growth of forest tree seedlings," submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree, Doctor of Philosophy. Project 612 of the Iowa Agricultural Experiment Station, Ames, Iowa.

²The author is indebted to Dr. W. E. Loomis, Prof. G. B. MacDonald and Dr. W. H. Pierre of the Iowa Agricultural Experiment Station for encouragement, advice and assistance during the prosecution of this work. Dr. J. E. Sass of the Botany Department prepared some of the sections of mycorrhizae and took some of the photomicrographs.

³The literature on this subject has been extensively reviewed by Kelley (20), Hatch (15) and Rayner (35).

element (15, 43) or of water (8), and to enable the plants to absorb carbohydrates (12, 13, 48) and organic nitrogen (28). In addition, a theory of nitrogen fixation by mycorrhizal fungi has been advanced.

The fact that conifers will grow well in some soils, apparently without the aid of mycorrhizae, has led to the belief that these fungi are not important for growth and are essentially weak parasites (25, 27, 40). Another view (5) is that mycorrhizal fungi, although parasitic, may indirectly benefit the host by releasing nutrients and other substances from organic matter.

More recently the view has been presented that good tree growth and the formation of mycorrhizae are intimately bound up with the chain of reactions characteristic of some types of organic matter decomposition and humus formation. According to Rayner (37), some soils accumulate substances which prevent activity of mycorrhizal fungi. In these soils tree growth is very poor. Similarly Young (48) has noted that the mycorrhizae of some pines were abnormal on sandy soils low in organic matter, and that on these soils, the fused needle disease was widespread. Where mycorrhizal fungi are active, Rayner (36, 37) noted that tree roots were often stimulated before mycorrhizae were formed and she concluded that introduction of active mycorrhizal material to an unfavorable soil "leads directly to the production of growth-promoting substances in the substrate." In a later experiment, Rayner (39) found that conifer seedlings were stimulated more by addition of certain composts to the soil than by addition of nutrient salts equivalent to those found in the composts. Lindquist (23) has shown that some fungal extracts stimulated conifer seedlings while extracts from other fungi were toxic.

There is some evidence that phosphorus plays an important role in the fungus-root relationship. Melin (28) showed that mycorrhizal fungi were greatly stimulated by root excretions containing phosphatides. In Australia (22) poor growth of pines was shown to be due to a phosphorus deficiency, and Young (48) presented an hypothesis regarding the role of phosphorus in relation to mycorrhizae and tree growth. According to his hypothesis mycorrhizae may furnish trees with carbohydrates. The addition of phosphorus to a soil low in this element results in increased phosphatide secretion from tree roots and increased growth of ground cover vegetation. Mycorrhizal fungi are stimulated by the excreted phosphatides and having additional vegetable detritus on which to work, are able to supply trees with significant quantities of carbohydrates. It is doubted, however, if carbohydrate nutrition is of importance on well aerated mineral soils where light is adequate for growth.

The phosphorus nutrition of mycorrhizal and non-mycorrhizal pines has not been widely investigated on a quantitative basis. Mitchell (30), however, has shown that white pine in sand

cultures grew best at phosphorus concentrations of 300-350 ppm. Conversely, work with apple (3) and peach (9) seedlings in sand cultures showed best growth at phosphorus concentrations of 4 ppm. With various herbaceous crop plants (33, 44) maximum growth has been obtained in solution cultures where phosphorus was maintained at levels of 1.0 ppm. or below. If Mitchell's data are representative of non-mycorrhizal pines, it is evident that conifers present a special problem in phosphorus nutrition.

In central Iowa some difficulties have been encountered in attempting to grow conifer seedlings in a nursery on a prairie soil. Inability to obtain satisfactory seedling growth apparently has been due to absence of mycorrhizae and poor phosphorus nutrition. This bulletin presents a description of difficulties encountered at the nursery and some experiments carried out to ascertain the causes of poor growth. The nutrition of pine seedlings is discussed in relation to factors peculiar to this nursery.

EXPERIMENTAL

The Iowa State Forest Nursery is located on an O'Neil sandy loam soil which occupies a high terrace in Section 14, T. 83 N., R. 23 W., about 1 mile south and slightly east of Ames, Iowa. The surface soil varies in depth from about 8 to 13 inches, and prior to acquisition as a nursery site the tract had been used for agricultural crops. The surface soil is dark brown to greyish brown in color, has a pH of from 5.8 to 6.5 and is underlain with a sandy gravel stratum at depths ranging from about 20 to 30 inches. Analyses showed this soil to contain on a per acre⁴ basis 2000 pounds total nitrogen, 40 pounds dilute acid-soluble phosphorus, and 1600 pounds of exchangeable calcium.

During the spring of 1937, the first crops of conifers were seeded in the nursery. The species included northern white pine, *Pinus strobus* L.; ponderosa pine, *Pinus ponderosa* Dougl.; Virginia pine, *Pinus virginiana* Mill.; and Japanese red pine, *Pinus densiflora*, Sieb. and Zucc.; all of which were mulched with pine needles from a vigorous 14-year old plantation of white and red pines. Other conifers seeded but not mulched with pine needles included red or Norway pine, *Pinus resinosa*, Ait.; Austrian pine, *Pinus nigra*, Arnold; Douglas fir, *Pseudotsuga taxifolia*, Brit.; and Norway spruce, *Picea Abies* Karst.

Following germination, good stands of all species except Austrian pine were obtained, and seedling development was normal until approximately Aug. 1 when the first fascicled needles began to appear. At this time it was noted that the beds which had been mulched with pine needles were spotted in appearance, and

⁴An acre of soil 6¾ inches deep is considered to contain 2 million pounds of soil materials.



Fig. 1. Two-year-old non-mycorrhizal Douglas fir on O'Neil soil at State Forest Nursery — 1938.

seedling growth was irregular. In certain areas of these beds the seedlings continued to make vigorous growth, while in other areas they were stunted and off-color. The seedlings of species not mulched with pine needles were uniformly stunted. As the season progressed, the difference between the good and poor spots in any bed became greater. In late September the stunted seedlings began to turn brown or reddish purple in color while the vigorous seedlings retained a normal green color. At the end of the growth period the vigorous seedlings were approximately twice as large as the stunted ones.

Upon examining the seedlings from the good and poor spots in any bed it was found that the vigorous seedlings invariably possessed an abundance of ectotrophic mycorrhizae, while stunted seedlings possessed few or none. This relationship was true of all seedlings from beds mulched with pine straw and was most pronounced with Virginia and Japanese red pines. The seedlings from beds of Austrian and red pine, Douglas fir and Norway spruce, which did not receive the needle mulch, were uniformly poor and none possessed observable ectotrophic mycorrhizae.

In the winter many of the non-mycorrhizal seedlings in the mulched beds died. During the second growing season, however, the size of the spots supporting vigorous seedlings increased, and

many of the off-color seedlings of the previous year now regained their greenness and began to make good growth. At the end of the second season all except a few of the spots previously showing stunting contained seedlings of good vigor. Size differences were still evident but all plants were making satisfactory growth.

In the beds not originally mulched with pine straw, winter mortality was very noticeable. In late spring of the second season,

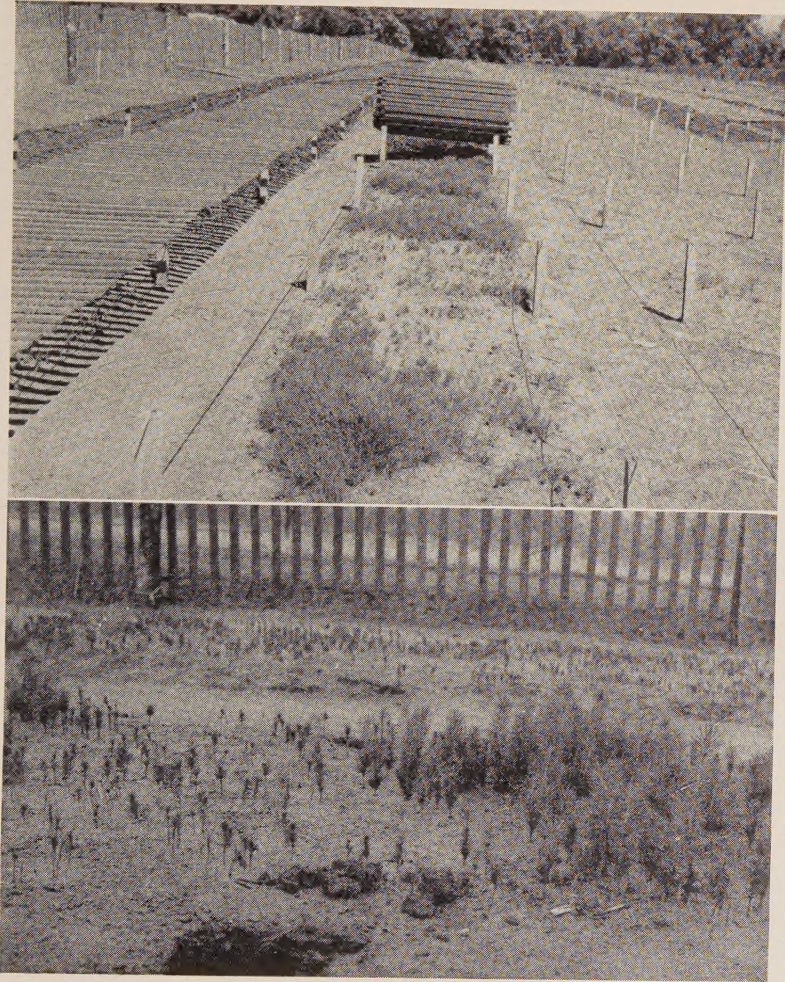


Fig. 2. Large mycorrhizal seedlings and small non-mycorrhizal seedlings of red pine (above) and Norway spruce (below). State Forest Nursery. (1938.)

however, a few small spots of vigorous seedlings appeared, and upon examination these seedlings were found to have mycorrhizae. These spots enlarged slowly during the growing period, but by the end of the second season approximately 90 percent of the Douglas fir seedlings, and only a slightly lower percentage of the Austrian pine, red pine and spruce were dead. The total loss in this instance was approximately 350,000 2—0⁵ seedlings. The condition of the Douglas fir, the red pine and the Norway spruce seedlings is illustrated in figs. 1 and 2.

INOCULATION OF NURSERY SEEDBEDS

To ascertain if the pine needles used to cover the seedbeds at the State Forest Nursery were a source of mycorrhizal fungi, inoculation of new coniferous seedbeds was attempted. In the spring of 1938 duff and humus-rich top soil were obtained from the plantation which had furnished the pine straw used to cover the 1937 seedbeds. It had been ascertained first that the trees in this plantation bore mycorrhizae. This soil was applied at the rate of 1 bushel per 250 sq. ft. of seedbed and was raked in to a depth of about 2 inches.

The bed was then sown to Scots pine, *Pinus sylvestris* L. The author was not able to observe the beds again until the first part of September, 1938. At this time striking differences were observed between plants from inoculated and uninoculated soil. These differences are illustrated in fig. 3. At the end of the second growing season, in 1939, many of the uninoculated seedlings were dead while those that remained alive averaged less than 2.0 inches in height. At this same time the inoculated seedlings, where competition was not excessive in the bed, reached an average height of about 7 inches.

Similarly, inoculation of seedbed soil with this same duff was attempted for other species. These species included white pine; jack pine, *Pinus banksiana* Lamb; and Douglas fir. The results obtained were similar to those obtained with Scots pine. In all cases a marked stimulation of growth occurred and mycorrhizae appeared in the inoculated but not in the uninoculated plots.

NUTRIENT ABSORPTION BY INOCULATED AND UNINOCULATED PINE

Since it has been observed (14, 15, 31) that mycorrhizae may enable coniferous seedlings to increase their absorption of some plant nutrients, a study was made of the nutrient content and root development of mycorrhizal and non-mycorrhizal pines from the State Forest Nursery. This work was started in the fall of 1937 and completed in the spring of 1938. 1—0 Virginia pine stock

⁵ In this designation the first digit represents the number of years in the seedbed; the second the number of years in the first transplant bed.

was used. A block of soil containing over 200 seedlings was removed from each of a mycorrhizal and non-mycorrhizal spot in one of the seedbeds. These blocks were within 10 feet of each other in the bed, the pH of the soil varied from 6.0 to 6.2, and from all outward appearances the soils in the two blocks were similar. The soil was carefully washed from the seedlings, and damaged plants at the edges of the blocks were discarded. From among the uninjured seedlings, a sample of 20 was chosen at random from each block for a study of the mycorrhizae and of seedling development. The remaining seedlings were used for chemical analyses.

The 20 mycorrhizal and the 20 non-mycorrhizal seedlings were examined externally to determine the nature of the absorbing roots, and counts were made of the number of mycorrhizal



Fig. 3. One-year-old Scots pine on O'Neil soil. Inoculated (above) and uninoculated (below).

TABLE 1. AVERAGE HEIGHT GROWTH AND ROOT DEVELOPMENT OF MYCORRHIZAL AND NON-MYCORRHIZAL *PINUS VIRGINIANA* SEEDLINGS.

Sample	Number of seedlings	Height growth		Short roots and mycorrhizal development*		
		Root collar to cotyledons (inches)	Cotyledons to bud (inches)	Total (inches)	Number mycorrhizal short roots	Number non-mycorrhizal short roots
Mycorrhizal	20	1.34±.05	1.36±.08	2.70±.09	350±28	321±22
Non-mycorrhizal	20	1.15±.06	0.85±.04	2.00±.06	7±2.1	297±15
Difference		0.19	0.51	0.70	343	24
T values		2.65†	5.86‡	6.3‡	11.9‡	0.88

* Root classification of Hatch and Doak (16).

† Significant.

‡ Highly significant.

TABLE 2. WEIGHT AND NUTRIENT CONTENT OF MYCORRHIZAL AND NON-MYCORRHIZAL SEEDLINGS OF *PINUS VIRGINIANA* GROWN AT THE STATE FOREST NURSERY.

Sample	Number of seedlings	Weight av. plant green (mgs.)	Weight av. plant dry (mgs.)	Nitrogen content		Phosphorus content		Potassium content	
				Percent dry weight	Average per plant (mgs.)	Percent dry weight	Average per plant (mgs.)	Percent dry weight	Average per plant (mgs.)
Mycorrhizal	25	1228	322	1.79	5.76	0.178	0.57		
	25	1216	307	1.72	5.28	0.182	0.56		2.06
	3	1232	329	1.80	5.92	0.186	0.61		2.14
	4	1246	333	1.81	6.03	0.192	0.64		2.30
	Average	1230±6.1	323±5.7	1.78±.019	5.75±.17	0.184±.003	0.60±.02	0.64±.015	2.17±.07
Non-mycorrhizal	50	626	165	1.85	3.05	0.089	0.15		
	50	566	148	1.85	2.74	0.092	0.14		1.07
	50	574	147	1.85	2.75	0.099	0.15		0.95
	32	600	150	1.96	2.94	0.110	0.16		0.93
	Average	592±13.6	152±4.2	1.88±.027	2.87±.08	0.097±.005	0.15±.004	0.63±.013	0.96±.04
Differences of averages		639	171	2.67*	2.88	15.8**	0.45	1.62	1.21
T values		42.9**	23.9**		15.8**		28.1**		15.9**

* Significant.

** Highly significant.



Fig. 4. One-year-old Virginia pine seedlings from O'Neil soil. Non-mycorrhizal (left) and mycorrhizal (right).

branches or tips and of the non-mycorrhizal short roots. The root classification of Hatch and Doak (16) was used. The same seedlings were used for the following measurements of height growth: From root collar to top of cotyledonary growth, from top of cotyledonary growth to the bud, and total height. The growth from the cotyledons to the bud was believed most useful in evaluating height growth response associated with mycorrhizae, because the initial growth effects due to the seed were probably less evident in this latter growth. Large seeds contain more food and essential nutrients (1, 30) and seedling yield has been shown to vary with seed size and weight (1, 24, 83).

The seedlings used for chemical analyses were counted and green and dry weights determined. The ground tissue was then analyzed for total nitrogen, phosphorus and potassium by the methods of the Association of Official Agricultural Chemists (2).

The results of all comparisons are presented in tables 1 and 2. Figures 4 and 5 illustrate the differences between mycorrhizal and non-mycorrhizal seedlings. The data are all clear cut, and there was little overlapping in the ranges displayed by individual measurements. Statistical significance was determined in all cases by the methods outlined by Snedecor (41). From these data, the following points can be made:

1. Green and dry weights of mycorrhizal plants were double those of the non-mycorrhizal plants.

2. Total height of mycorrhizal plants was 35 percent greater, and height growth from cotyledons to the bud 60 percent greater than that of the non-mycorrhizal seedlings.

3. The mycorrhizal plants were slightly, but significantly longer (17 percent) from root collar to cotyledons than the non-mycorrhizal plants.

4. The average mycorrhizal plant had well over 600 absorbing short roots and mycorrhizal tips or branches while the non-mycorrhizal plants had only slightly over 300.

5. The number of non-mycorrhizal short roots on mycorrhizal and non-mycorrhizal plants was about the same.

6. The extra absorbing root tips on the plants from inoculated soil were thus mycorrhizal and can be thought of as extra root tips formed as a result of the mycorrhizal stimulus.

7. Mycorrhizal plants contained (totals per plant) twice as much nitrogen and potassium and four times as much phosphorus as non-mycorrhizal plants.

8. On a percentage of dry weight basis, the seedlings with mycorrhizae contained twice as much phosphorus as non-mycorrhizal plants but there was little difference in the contents of nitrogen and potassium.

The above data indicate that mycorrhizae, or the conditions which permit formation of mycorrhizae, were directly stimulating to root growth and activity and that plants thus stimulated were capable of absorbing increased quantities of phosphorus — an element which was apparently limiting growth in this soil.



Fig. 5. Root systems of 1-0 Virginia pine from O'Neil soil. Non-mycorrhizal (left), mycorrhizal (right).

RESPONSES OF JACK PINE TO NITROGEN
AND PHOSPHORUS

In view of the poor color of the non-mycorrhizal seedlings started in 1937, and other indications of nutrient deficiencies, direct additions of nitrogen and phosphorus to conifer seedlings were tested. These experiments were started in the spring of 1938 before the data obtained with Virginia pine had been evaluated. Jack pine was chosen for the experiments because seed of Virginia pine was not available and because jack pine normally makes rapid growth, once past the cotyledon stage. If seedlings inherently grow slowly any fertilizer effects are partially masked by slow growth and inherent variability.

The experiments started in the spring of 1938 were carried out in cypress boxes about 11 inches deep and 10 inches square, with a capacity of slightly less than two-thirds of a cubic foot. The soil used was O'Neil sandy loam obtained from the surface of an eroded spot in a field next to the nursery and probably represents the A_3 or B_1 horizon, although the field had been cropped to corn and sorghum for the 2 years prior to collecting the soil, and horizons were mixed. This eroded soil was chosen to get material which from appearances was lower in total nitrogen than the surface soil of uneroded spots. Analyses showed it to contain approximately the same quantity (18.5 ppm.) of dilute acid-soluble phosphorus as was found in the nursery surface soil.

After the soil had been thoroughly mixed it was measured out box by box and the fertilizers mixed completely with all the soil in each box. Four treatments were used: (1) Check, (2) high nitrogen-low phosphorus, (3) high nitrogen-high phosphorus, and (4) low nitrogen-high phosphorus. All boxes received a basic application of 360 pounds K_2O per acre as potassium sulphate. Rates of application were calculated on a volume basis. Nitrogen was added as ammonium sulphate and phosphorus as mono-calcium phosphate. The rates of application of nitrogen and phosphorus are shown in table 3. All treatments were replicated five times.

Jack pine seeds were planted in each box, and the boxes were then mulched with reeds and sedges and set on planks in a small snow fence enclosure at the nursery. After emergence the seedlings were kept shaded until Aug. 1, at which time they were considered hardy enough to stand full exposure. Throughout the growing seasons they were watered to optimum. Late in November, 1938, all plants were mulched to prevent winter injury.

During the spring of 1939, the mulch was removed and the boxes refertilized with nitrogen equal to that originally added, and phosphorus equivalent to one-half the original quantity. Refertilization was decided upon after tests made during the previous late summer indicated that most of the nitrogen either

TABLE 3. GROWTH OF JACK PINE IN RELATION TO SEVERAL FERTILITY TREATMENTS
ON O'NEIL SANDY LOAM.

Treatment*	Box no.	No. trees	Height growth — inches			Dry weight — grams			Green weight grams
			1938	1939	Total	Top	Root	Total	
Check	102	2	0.9	1.0	1.9	0.29	0.18	0.47	0.8
	103	9	4.7	5.0	9.7	1.02	0.72	1.74	7.4
	104	10	6.4	5.4	11.8	1.26	0.96	2.22	4.1
	105	3	1.4	0.9	2.3	0.42	0.43	0.85	1.7
	Total av.	24	13.4	12.3	25.7	2.99	2.29	5.28	14.0
Inoculated check	101	13	0.6	0.5	1.1	0.12	0.10	0.22	0.6
	av.	13	49.7	16.8	66.5	20.28	16.37	36.65	97.0
	116	8	38.9	17.7	56.6	23.87	20.66	44.53	116.7
	117	12	26.7	24.4	51.1	18.26	14.19	32.45	75.8
	118	7	14.8	11.7	26.5	7.40	12.02	19.42	40.7
High nitrogen (144 lbs. NH_3/A) Low phosphorus (43 lbs. $\text{P}_2\text{O}_5/\text{A}$)	119	12	38.5	26.2	64.7	30.72	26.12	56.84	131.2
	120	10	21.0	23.1	44.1	11.87	13.95	25.82	49.3
	Total av.	49	139.9	103.1	243.0	92.12	86.94	179.06	413.7
	111	12	48.4	39.7	88.1	42.44	35.44	77.88	182.3
	112	11	46.8	39.8	86.6	45.18	28.11	73.29	176.6
High nitrogen (144 lbs. NH_3/A) High phosphorus (172 lbs. $\text{P}_2\text{O}_5/\text{A}$)	113	11	48.0	32.2	80.2	38.11	31.86	69.97	193.0
	114	9	38.7	20.6	59.3	32.61	19.20	51.81	138.4
	115	9	41.3	26.5	67.8	33.49	23.77	57.26	144.6
	Total av.	52	223.2	158.8	382.0	191.83	138.38	330.21	834.9
	106	10	38.0	26.5	64.5	35.68	27.54	63.22	128.1
Low nitrogen (36 lbs. NH_3/A) High phosphorus (172 lbs. $\text{P}_2\text{O}_5/\text{A}$)	107	8	41.5	23.3	64.8	29.32	28.42	57.74	144.4
	108	10	56.3	24.2	80.5	51.11	18.47	69.58	200.1
	109	12	49.0	31.0	80.0	27.05	24.90	51.95	120.2
	110	10	28.4	32.8	61.2	35.60	26.74	62.34	147.0
	Total av.	50	213.2	137.8	351.0	168.66	126.07	294.73	739.8
	av.	50	4.3	2.8	7.0	3.73	2.52	5.89	14.9

* All boxes received potassium at the rate of 360 pounds K_2O per acre.

had been used or had been leached from the soil. At this time nitrogen was added as ammonium nitrate instead of the sulphate. Phosphorus was applied as a solution of mono-calcium phosphate,



Fig. 6. Two-year-old jack pine seedlings grown on O'Neil soil at different levels of nitrogen and phosphorus. Left to right: check, N-1 and P-3, N-3 and P-3, N-3 and P-1.

the half application being dissolved in one liter of water so as to obtain maximum penetration of the phosphate. Following refertilization the seedlings in each box were thinned to 12 per box to avoid serious competition among the plants.

During the first growing season several of the seedlings in each of the check boxes died. About Sept. 15 of the first season it was first noted that the seedlings in one of the check boxes were definitely superior to those in the other four check boxes. This difference increased during the fall and was very pronounced throughout the second growing season. In the second season it was noted also that considerable variability occurred among the replications of plants receiving the low phosphorus-high nitrogen treatment. The good growth of the one check was later ascertained to be due to fortuitous inoculations with mycorrhizal fungi.

During the winter of 1940 this experiment was dismantled, the seedlings examined, and heights and green and dry weights obtained. These data are presented in table 3 and are illustrated in figs. 6 and 7. Data for analysis of variance are presented in table 4. From the data the following points are evident:

1. Jack pine grew very poorly or died on this soil when unfertilized or uninoculated.

2. Total dry weight increase of the high P and N treatment over the check was about 2800 percent.

3. The major part of the growth response obtained apparently was due to phosphorus.

4. Conclusive evidence of the importance of nitrogen could not be derived from this experiment, but indications were that nitrogen response was obtained, if at all, only after the phosphorus requirements had been met.

5. The similarity between the growth of the inoculated check (box 101) and the low phosphorus-high nitrogen treatment suggests that, if inoculated from the first, mycorrhizal seedlings would grow as well as those of the above fertility treatment.

Observation of the seedlings from uninoculated fertilized soil showed that they possessed a great abundance of light colored, apparently active absorbing root tips. These tips were definitely swollen, were sometimes branched, and superficially resembled mycorrhizal roots. Study under a low power (100X) microscope, however, failed to disclose any fungal mantle, and it was tentatively concluded that these roots were either non-mycorrhizal or else were endotrophic mycorrhizae. When some of these roots were later sectioned and studied under higher magnifications they were found to be mycorrhizae of the common type, but differed

from those found on the inoculated plants in that the fungal infection was strictly intercellular, the hyphae much smaller in diameter, and the mantle more compact.

To compare the phosphorus response of pine seedlings with another type of plant, oats were grown on O'Neil soil treated with various levels of nitrogen and phosphorus. On this soil where jack pine showed a 2800 percent response, most of which was due to phosphorus, oat plants showed a 700 percent response to nitrogen and an 80 percent response to phosphorus. These results suggest that there is a vast difference, either in the feeding power or in the requirements of pines and oats as far as phosphorus and nitrogen are concerned, and that without mycorrhizae pines acquire phosphorus with difficulty.



Fig. 7. Two-year-old jack pine seedlings from O'Neil soil. Non-mycorrhizal (left) and mycorrhizal (right).

TABLE 4. ANALYSIS OF VARIANCE OF HEIGHT GROWTH AND TOTAL DRY WEIGHT FOR 2—0 JACK PINE, NITROGEN-PHOSPHORUS SERIES, ON O'NEIL SOIL.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Height growth			
Total	18	123.08	37.01**
Treatments	3	111.03	0.80
Error	15	12.05	
Total dry weight			
Total	18	127.81	37.62**
Treatments	3	113.88	0.93
Error	15	13.93	

** Highly significant.

GROWTH OF JACK PINE ON SEVERAL SURFACE SOILS

Simultaneously with the experiment with nitrogen and phosphorus, jack pine was grown on several Iowa surface soils. The soils used were Webster silty clay loam obtained from the Agronomy farm of the Iowa State College, D-Clarion⁶ loam, and Ames silt loam. The Webster is a prairie soil, the Ames a forest soil obtained near the Ledges State Park, and the D-Clarion soil used was a degraded prairie soil obtained from the Agronomy farm. The setup for this experiment was similar to that for jack pine on O'Neil soil. All soils were left untreated. Data concerning these soils are presented in table 5.

The results of this experiment are presented in table 6 and illustrated in fig. 8. On the Webster soil which had a pH of 7.8 and an abundance of free calcium carbonate, the seeds germinated and the seedlings lived in a chlorotic stunted state for from 1 to 4 months and then died. None was alive in the late fall. On the Clarion and Ames soils, both of which were slightly acid and originally supported a mixed stand of oaks, hickory and elm, seedling growth was very good and in fact superior to the growth obtained on the fertilized O'Neil soil. All plants grown on D-Clarion and Ames soils possessed mycorrhizae and were vigorous and of a healthy color.

EFFECT OF SOIL INOCULATION AND FERTILIZATION ON THE ROOT SYSTEMS OF PINE

Microscopic studies were made of the root systems of pines to compare the effect of inoculation with that of fertilization. This work was done with jack pine grown in 1938-39 and white

⁶ D-Clarion refers to Clarion soil, originally prairie, which had been partially leached and degraded as a result of occupation by forest.

TABLE 5. SOME CHEMICAL CHARACTERISTICS OF THE SOILS USED TO GROW *PINUS BANKSIANA*.

Measurements	O'Neil	D-Clarion	Ames	Webster
pH	6.0	6.0	6.0	7.9
Total nitrogen (lbs./acre)	1900	3860	2453	5860
Dilute-acid-soluble phosphorus (lbs./acre)	37	68	65	139
Exchangeable calcium (lbs./acre)	1600	4960	2200	Alkaline soil Free CaCO ₃

pine grown during 1940-41. The fertilized plants studied received phosphorus, nitrogen and potassium.

A wide variety in the type of mycorrhizal roots and in the degree of infection was revealed by this study. Root tips from seedlings of jack and white pine from inoculated unfertilized O'Neil soil were (1) simple uninfected short roots, (2) ecto-

TABLE 6. GROWTH OF JACK PINE ON SEVERAL UNTREATED SURFACE SOILS AND ON FERTILIZED O'NEIL SUBSOIL.

Soil	Box no.	No. trees	Total height (inches)	Dry weight (grams)			Green weight (grams)
				Top	Root	Total	
Webster	161 to 165	All plants died during first season					
D-Clarion	131	11	80.0	66.5	35.2	101.7	254.1
	132	10	57.3	46.6	32.0	78.6	207.4
	133	10	76.3	55.0	52.1	107.1	256.2
	134	10	74.1	55.8	40.2	96.1	242.7
	135	12	74.3	59.5	35.0	94.5	233.0
	Total Av.	53	362.0 6.8	283.4 5.3	194.5 3.7	478.0 9.0	1193.4 22.5
Ames	146	13	77.8	36.3	20.4	56.8	138.9
	147	11	77.6	54.5	37.6	92.1	231.6
	148	8	44.0	43.2	41.9	85.1	240.7
	149	10	83.4	52.7	30.8	83.6	220.2
	150	10	66.8	72.2	45.2	117.4	275.9
	Total Av.	52	349.6 6.7	258.9 5.0	175.9 3.4	435.0 8.4	1107.3 21.3
O'Neil* NPK	111	12	88.1	42.4	35.4	77.9	182.3
	112	11	86.6	45.2	28.1	73.3	176.6
	113	11	80.2	38.1	31.9	70.0	193.0
	114	9	59.3	32.6	19.2	51.8	138.4
	115	9	67.8	33.5	23.8	57.3	144.6
	Total Av.	52	382.0 7.3	191.8 3.7	138.4 2.7	330.3 6.4	834.9 16.1

* The O'Neil soil received 360 lbs. K₂O, 144 lbs. NH₃, and 172 lbs. P₂O₅ per acre.

trophic mycorrhizae, or (3) ectendotrophic mycorrhizae (those possessing both inter- and intra-cellular infection). On white pine two types of fungal mantles were observed, one of which was



Fig. 8. Two-year-old jack pine seedlings on several soils. Left to right: D-Clarion, O'Neil + NPK, Ames. Below: O'Neil, check.

relatively thin and light brown in color while the other was heavy and dull grey. In both cases branching was dichotomous. Among the mycorrhizae there was no sharp dividing line between ecto- and ectendotrophic types, and apparently the same fungus was found inside as well as between the cells. If this is the case, then differences in mycorrhizal type in inoculated soil represented differences in degree of infection and perhaps in relative vigor of host and fungus. In instances where intracellular infection was severe, the walls of cortical cells were often irregular and appeared partially broken down. Sometimes one side of a root possessed intercellular hyphae while the other side was uninfected. The non-mycorrhizal short roots possessed small, slightly swollen, light brown absorbing tips while both of the mycorrhizal types exhibited considerable hypertrophy, and the light colored tips were four or more times longer than those of the uninfected roots.

As previously indicated, application of phosphorus to uninoculated O'Neil soil resulted in good growth of seedling pines and in the formation of an extensive absorbing system with enlarged short roots. Microscopic sectioning of these enlarged roots showed them to be ectotrophic mycorrhizae. They possessed both a well developed mantle and the Hartig net, but none had intracellular infection. These mycorrhizae differed from those on inoculated plants in that the intercellular net often appeared to be composed of two or more mycelial strands each of which was only about half as large in diameter as those from inoculated plants. The strands of mycelium making up the mantle were

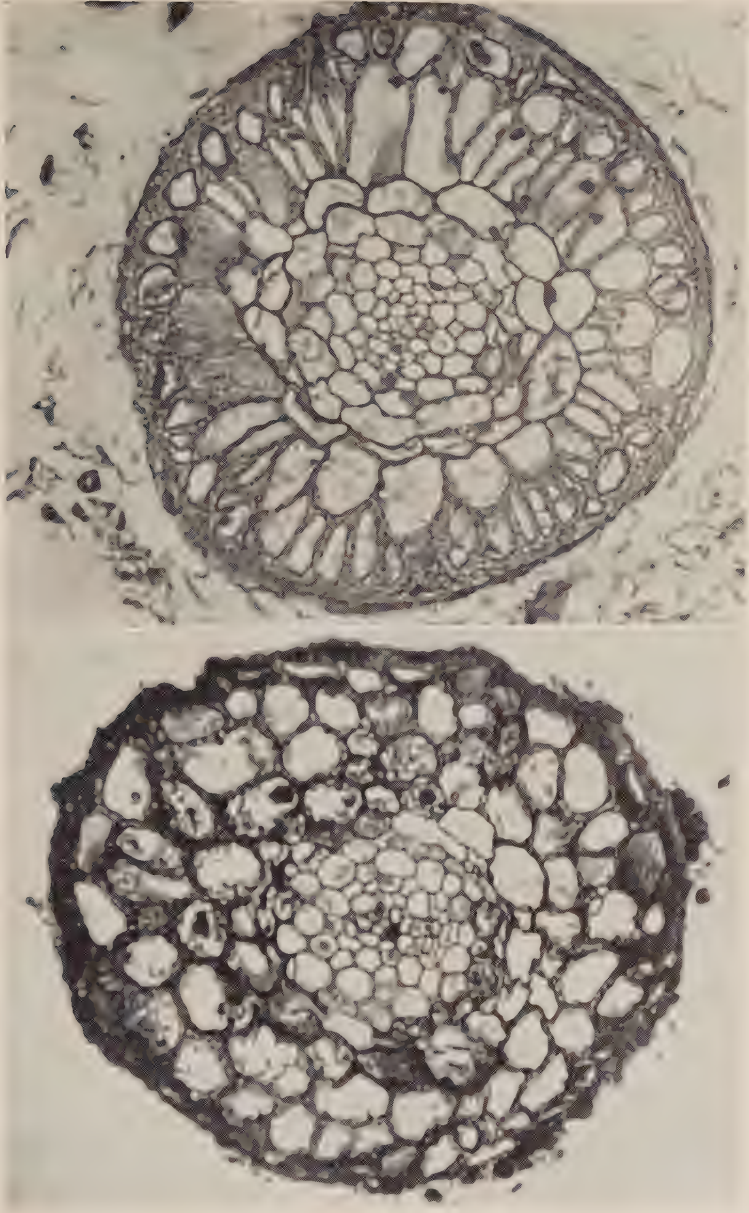


Fig. 9. Pine mycorrhizae. Above: Jack pine from fertilized uninoculated soil. (184X). Below: White pine from inoculated unfertilized soil. (144X).

also small in diameter, and the mantle was much more compact than that found on the inoculated seedlings. Because of the compactness of the mantle and the small diameter of the mycelial threads, the mycorrhizal character of these roots was not detected with external examination using a low power microscope (100X), and at first they were thought to be either endotrophic mycorrhizae or uninfected roots. The fertilized uninoculated plants had about the same proportion of uninfected roots as was found on inoculated unfertilized plants.

In view of these results it seems probable that the O'Neil soil originally contained fungi capable of forming mycorrhizae, but that soil or plant conditions favorable to the formation of mycorrhizae were not obtained prior to fertilization with phosphorus. The mycorrhizal fungi from these roots have not been cultured and their identities are unknown, but in view of the facts presented and the differences in thickness of the mycelium, it seems reasonable to assume that at least two species were involved, one of which may have existed in a subdominant state in the unfertilized soil.

Several non-mycorrhizal short roots from plants neither fertilized nor inoculated were sectioned for microscopic examination. None of these roots showed intra- or inter-cellular infection and therefore cannot be considered pseudo-mycorrhizal. All of such roots were heavily impregnated with materials not found present in large quantities in mycorrhizal roots. Such roots were heavily cutinized and the cortex and endodermis were well filled with globules of resinous or gummy appearing material. Non-mycorrhizal roots commonly contained one less row of cortical parenchyma than mycorrhizal roots.

DISCUSSION

The data obtained for Virginia and jack pines, and observations on uncompleted studies with white pine and Douglas fir, support in general the mineral nutrition theory of mycorrhizae as developed by Stahl (43) and Hatch (15). According to this theory (15), mycorrhizal seedlings receive better nutrition because of (1) the greater number of absorbing short roots resulting from profuse branching of the mycorrhizal structure, (2) the greater surface area of the infected (mycorrhizal) short roots, (3) the delay in suberization of the cortex and endodermis, and (4) the great extension of the fungal mycelium through the soil resulting in increased surface for absorption. According to Hatch, fungi have greater capacities to acquire nutrients, especially those in organic combination, than do tree seedlings, and nutrients acquired by the fungi tend to move to the plant on a gradient.

The data obtained with Virginia pine show that there were twice as many absorbing tips on mycorrhizal as on non-mycorrhizal

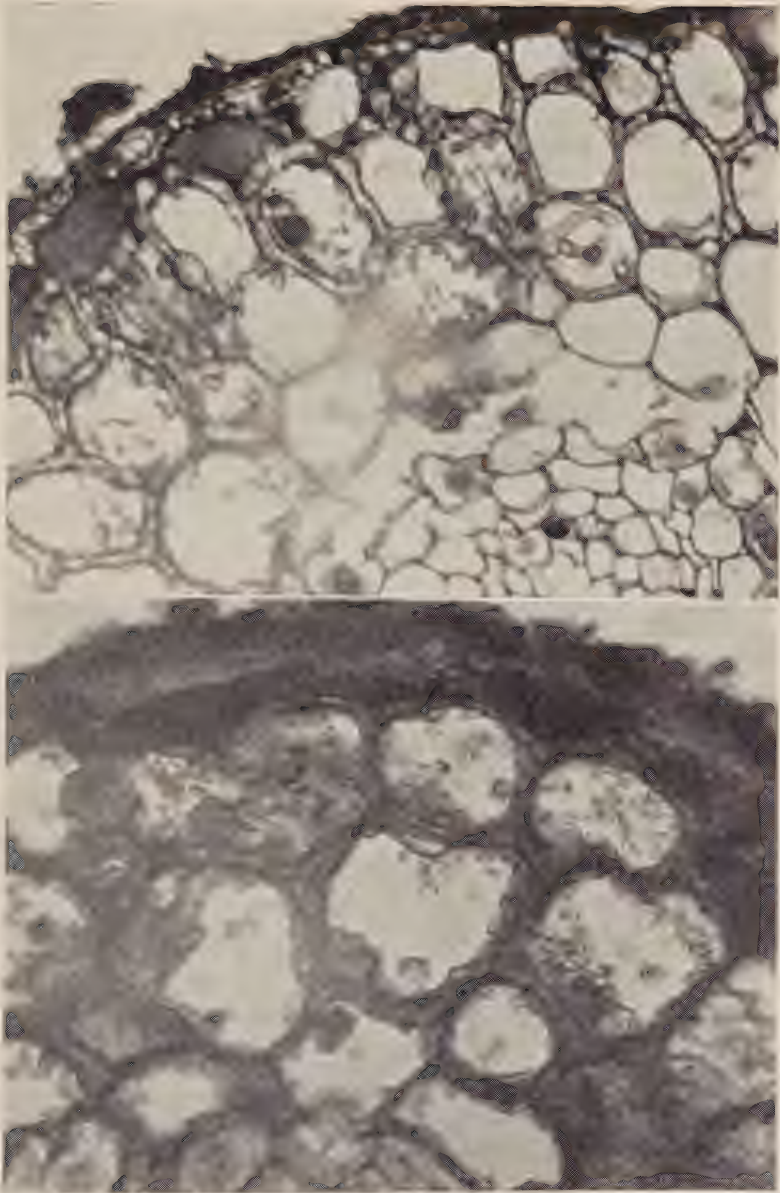


Fig. 10. Mycorrhizae of white pine grown on O'Neil soil. Above: From inoculated unfertilized soil. Below: From uninoculated fertilized soil. (325X).

seedlings. Because of short root hypertrophy and the greater number of absorbing short roots, the absorbing area of mycorrhizal plants was often 20 to 30 times greater than that of non-mycorrhizal plants. These data are supported by studies and observations on white pine and Douglas fir. Studies with white pine showed non-mycorrhizal roots to be highly differentiated to within a fraction of a millimeter of the tip, to contain a well suberized endodermis and a heavy external layer of cutin, and to have cortical cells heavily laden with resinous and gummy appearing materials. Conversely, mycorrhizal roots were poorly differentiated and appeared to be permeable for a distance of 2 millimeters from the tip.

Important differences in the phosphorus nutrition of mycorrhizal and non-mycorrhizal seedlings are observable in the absorption data for Virginia pine and the fertilizer experiments with jack pine. Mycorrhizal Virginia pine seedlings absorbed four times (twice as much on a dry weight basis) as much phosphorus as non-mycorrhizal plants. There was no increase in the percentage of nitrogen or potassium. Similarly, jack pine and other species of seedling conifers have shown remarkable responses to phosphorus fertilizers and practically none to nitrogen. These data do not support the original organic nitrogen theory of Melin (28) if the latter is used in the restricted sense that mycorrhizae are beneficial to plants only by enabling them to acquire an adequate supply of nitrogen, but it is possible that organic nitrogen in important quantities may be made available from peat and raw humus soils.

Comparison of the yields of jack pine and observations of white pine from fertilized but uninoculated soil and from inoculated but unfertilized soil suggest that either treatment was about equally beneficial. Furthermore, it has been shown that mycorrhizae developed on pines from uninoculated soil if phosphorus was applied, or on plants from unfertilized soil if coniferous humus was added. These facts tend to indicate, for the soils concerned, that phosphorus plays an important role in the formation of mycorrhizae, as well as in the growth of conifer seedlings, and that the two may be interdependent. The fact that mycorrhizae formed in large numbers on fertilized plants does not support the observations of Hatch (15) or Mitchell et al. (31) that abundance of mycorrhizae is inversely proportional to the fertility of the soil. These findings also tend to throw doubt either on the adequacy of autotrophic nutrition or on the ability of trees apparently well supplied with nutrients to ward off the mycorrhizal infection.

A number of workers on mycorrhizae have stated that phosphorus bearing root secretions stimulate the development of the fungous mycelium. Melin (28) found experimentally that plant secretions containing phosphatides caused increased growth

of mycelia, and Young (48) suggests that phosphorus-bearing substances from tree roots stimulate fungi to greater activity. In the present case the fact that phosphorus fertilization of jack and white pine resulted in formation of mycorrhizae on uninoculated soils might be advanced as evidence of the stimulating effect of phosphorus on mycorrhizal fungi. Contrarily, the fact that formation of mycorrhizae on Virginia pine was followed by increased absorption of phosphorus by seedlings could be used as evidence that phosphorus is not the only limiting factor and that growth stimulation of fungi might be due to some other factor. How (18) has shown that *Boletus elegans* was stimulated by a water soluble substance obtained from larch roots, and Steward (44) could not find evidence for the diffusion of phosphatides from living plant cells.

There is also evidence that the absorbing roots of conifer seedlings can be stimulated by fungi or their secretions. Rayner (36) (37) states that trees are stimulated before mycorrhizae are formed, and she attributes this stimulation to growth promoting substances deriving from fungal activity, and to liberation of nutrients needed by higher plants. Rayner (39) has found also that pines were stimulated more by composts than by nutrients equivalent to those present in the compost. Lindquist (23) found that spruce seedlings were stimulated by secretions from two unnamed mycelia and by that of *Mycelium radialis nigrostrigosum*, but were retarded by secretions from the parasitic soil fungus *M.r. atrovirens* and by a Danish strain of *Mucor ramannianus*. The fact that the short roots of Virginia, jack and white pines grown at the Ames nursery were obviously increased in both number and size by the mycorrhizal fungi is further evidence along this line. This evidence along with data from the literature strongly suggests that the initial stimulation of roots by fungi, and of fungi, by root secretions, can be more logically attributed to growth promoting substances than to better mineral nutrition.

The formation of mycorrhizae following phosphorus fertilization of uninoculated O'Neil soil is difficult to explain. The most obvious deduction is that the fungus concerned was different from that found in the coniferous humus used for soil inoculation, and that it was sub-dominant in the soil until phosphorus was added. The role of phosphorus in this instance might have been (1) to directly stimulate the fungus and enable it to attack the root at which time it could have stimulated the root, (2) to directly stimulate the root causing it to become less differentiated and more subject to later fungal attack or (3) to cause both of the above to happen simultaneously. Our data furnish no clues.

Considering the growth and absorption data as a whole, it is obvious, however, that the seedlings were not capable of obtaining adequate phosphorus unless either the concentration of soil phosphorus was high, or the roots were stimulated through

formation of mycorrhizae. Experience at the Ames nursery indicates that phosphorus applications equivalent to approximately 1000 pounds per acre 20 percent superphosphate produced the best seedling growth and that while inoculation was equivalent to at least a small phosphate application, heavy phosphorus fertilization with or without inoculation produced best growth. At the same nursery Young (47) found that jack pine responded through five steps to phosphoric acid equivalent to 1700 lbs. of 20 percent superphosphate per acre. On this same O'Neil soil containing approximately 1900 pounds of total nitrogen per acre, only a slight nitrogen response has been obtained and this only after the phosphorus requirement had been met.

Assuming an adequate quantity of an available form of phosphorus in the soil, failure to absorb this element might be due to inability of roots to absorb it and pass it through the root membranes (7), or to restriction of the absorbing area of the root (10). Rough estimates made in connection with the study of Virginia pine roots indicated that the surface area of absorbing roots of mycorrhizal plants was about 20 to 30 times larger than that of non-mycorrhizal plants. The active absorbing zone at the tips of non-mycorrhizal short roots appeared to be only about one-half of a millimeter in length, while those of the mycorrhizal roots averaged 2 millimeters or more. Prevot and Steward (34) state that the absorbing zone of barley roots grown in culture solutions is approximately 5 cm. in length. When one contrasts the fibrousness of most grass plant roots with that of trees and at the same time takes into consideration differences in absorbing area of root tips, it is easy to believe that a small total absorbing area might be an important factor in the failure of non-mycorrhizal pine tree roots to absorb phosphorus. Sommer (42) found an apparent relation between the extensiveness of the root system of plants and their ability to absorb phosphorus.

It is equally possible that non-mycorrhizal pine roots are unable to absorb phosphorus rapidly and transfer it across the protoplasmic membranes of the root. Hoagland (17) and other workers at the plant nutrition laboratory of the University of California have found that the rate of salt accumulation by plants is dependent upon the respiratory activity of root tissues. Crafts and Broyer (7) have suggested that the absorption and transfer of nutrients to the stele depends upon the maintenance of activity gradients across the cortex and that activity in plants with good nutrition depends upon gradients of oxygen and carbon dioxide. McGeorge and Breazeale (26) have found that liberation of carbon dioxide by roots is necessary for phosphate absorption from calcareous soils. Buehrer (4) postulates that ease of phosphate absorption is dependent upon the isoelectric point of the protoplasmic micelles of the root in relation to the pH of the surrounding soil solution. When the pH of the surrounding

solution is higher than the pH at this isoelectric point, the micelles become negatively charged and phosphate is absorbed with difficulty or not at all. The lower the isoelectric point of the protoplasmic micelles, the lower must be the pH of the surrounding soil solution if phosphate is to be rapidly absorbed.

Whether some or all of the above factors are important in the phosphorus nutrition of pine can be ascertained only by future experiments. It is conceivable that root activity of non-mycorrhizal pines, in soils with unbalanced nutrients, would be on such a low level as to preclude adequate nutrition. Secretions from mycorrhizal fungi might act as stimulants to root respiration. Our own data as well as data recently reported in the literature show that mycorrhizal fungi increase rather than retard growth of short roots. Increased respiration and activity would naturally be part of this more rapid growth, and according to Hoagland's conception would result in greater salt accumulation. Greater carbon dioxide liberation by rapidly respiring roots would tend to decrease the pH of the soil solution immediately adjacent to the roots and according to Buehrer would result in more rapid phosphate absorption.

The above hypothesis, however, would not explain the apparent ability of non-mycorrhizal seedlings to make satisfactory growth on some soils. Such a hypothesis does not explain the ability of jack pine seedlings from uninoculated fertilized O'Neil soil (assuming root stimulation to occur before mycorrhizae are formed and before fungal secretions reach the roots) to develop a more active root system, and it leaves out of consideration the stimulating effect that phosphorus is known to exert on the roots of other types of plants. The stimulation of roots by phosphorus alone can best be explained by the assumption that this element is connected with the respiratory mechanism in some such a way as Meyerhof and Kiessling (29) have conceived. (For more complete treatment of this subject see Werkman and Wood (46).) According to such an hypothesis, non-mycorrhizal seedlings in soils not rich in phosphorus would be inherently incapable of absorbing phosphorus at a rate rapid enough for best root growth. Increasing the concentration of soil phosphorus, however, would result in an initial increase of absorption, which by stimulating respiration might markedly increase further absorption and thus total growth. In the case of mycorrhizal seedlings, the transfer of phosphorus bearing, or other growth stimulators, from the fungus to the tree root would result in greater surface area and greater activity of absorbing roots, which presumably would be followed by increased absorption. According to the foregoing postulates, it would be impossible to separate the effects of increased surface area of roots from the effects of increased root activity since each affects the other.

It has been noted that the jack pine seedlings failed on the

alkaline, calcareous Webster soil. Many of the seedlings that emerged damped off, and the remainder made very little growth. As the season progressed chlorosis became more and more evident and seemed to develop progressively in tissue of any given age. Chapman (6) working with shortleaf pine, found that seedlings did not survive in sand cultures if the pH exceeded about 6.5 or the soluble calcium exceeded 500 ppm. He suggests that failure to survive is not due to a deficiency of such minerals as iron or phosphorus since his plants died while in the seedling stage at which time there would be an adequate supply of necessary nutrients within the cotyledons. Where there is an abundance of soluble calcium in the soil, Chapman suggests that toxicity is due to changes in the degree of permeability of the protoplasm and possibly disorganization of the protoplasm. Howell (19) attributed poor growth of ponderosa pine in alkaline culture solutions to chlorosis caused by the inability to absorb or translocate iron. The fact that chlorosis did develop in jack pine seedlings that survived several months suggests a nutrient deficiency, possibly arising as a result of the excess of calcium carbonate in the soil or the high pH value. Under these soil conditions it is probably true that phosphorus absorption was limited. Lack of mycorrhizae and consequent reduction in the size of the absorbing system undoubtedly contributed to poor absorption from this soil.

SUMMARY

At a new forest tree nursery established on O'Neil sandy loam soil pine seedlings failed to grow unless they developed mycorrhizae. Formation of mycorrhizae and satisfactory growth followed inoculation of the nursery soil with duff and humus from a healthy pine plantation.

Comparison of mycorrhizal and non-mycorrhizal Virginia pine seedlings revealed that plants with mycorrhizae had much larger root systems and more and larger absorbing root tips than plants without mycorrhizae. Infection with mycorrhizal fungi increased rather than retarded growth of short roots. Similarly, chemical analyses of the seedlings showed that mycorrhizal seedlings contained four times as much phosphorus as non-mycorrhizal plants. On a percentage dry weight basis mycorrhizal plants absorbed twice as much phosphorus, and there was no difference in the absorption of nitrogen and potassium.

Fertilizer experiments using jack pine and other species of conifers on uninoculated soil showed that good growth followed phosphorus fertilization, while little or no response was obtained with nitrogen. Seedling growth following phosphorus fertilization was approximately equal to that obtained when unfertilized soil was inoculated with humus containing mycorrhizal material.

Short roots of fertilized white pine seedlings from uninoculated soil were mycorrhizae of the ectotrophic type and possessed a very compact mantle composed of fine mycelia. Short roots from seedlings on inoculated unfertilized soil were usually ectendotrophic mycorrhizae with coarse hyphal threads. Two species of fungi may be involved in the formation of mycorrhizae on white pine, one of which may have been subdominant in the original nursery soil and incapable of forming mycorrhizae without phosphorus fertilization. Because of a compact mantle and fine hyphae the mycorrhizal nature of many short roots could not be determined without sectioning.

Jack pine was found to be mycorrhizal and to make satisfactory growth when grown on two forest soils taken from oak-hickory stands. This species failed completely when grown in an alkaline prairie soil of the Webster series.

Factors possibly of importance in the phosphorous nutrition of pine are discussed. It is suggested that failure of non-mycorrhizal pines on O'Neil soil may be due to a low level of root respiration, and that root respiration and activity may be stimulated by secretions from mycorrhizal fungi and by phosphorus.

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